The cross-over classes are the red-bar and white-eyed male flies. The percentage of crossing-over being 2,172, divided by 4,893, and the quotient, multiplied by 100, which gives 44.4 per cent. The difference, then, in the cross-over values when the linked factors entered in different ways was but 1.1 per cent., which does not seem to be a signicant difference.

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ON COUNTING CHROMOSOMES IN POLLEN-MOTHER CELLS

THE genetic study of hybrids between species with different chromosome numbers and of certain mutants requires the counting of many chromosome groups and raises the question of the best technique for the purpose. The staining qualities of aceto-carmine, which has long been used for preliminary work, especially by zoologists, are considerably improved by a trace of ferric salt. (Bolles Lee, in his well-known manual, gives formulæ for iron carmine; but this has no advantage for sections over iron hæmatoxylin.)

Iron Aceto-carmine 1.—Ordinary aceto-carmine is prepared by heating a 45 per cent. solution of glacial acetic acid to boiling with excess of powdered carmine, cooling and filtering. The young anthers are teased out with steel blades or needles in a drop of this until it changes slightly toward bluish red. An excess of iron spoils the preparation. Anther remains are removed, and a large thin coverglass (22 by 50 mm.) applied, using the minimum of liquid. The edges are sealed with vaseline. The preparation, if there is no excess of iron, may improve for a day or two.

Iron Aceto-carmine 2.—To a quantity of aceto-carmine a trace of a solution of ferric hydrate dissolved in 45 per cent. acetic acid is added until the liquid becomes bluish red, but no visible precipitate forms. An equal amount of ordinary aceto-carmine is then added. The anthers are teased out with nickel instruments. If the stain is too dark, more aceto-carmine is to be supplied. It may be diluted with 45 per cent. acetic.

Iron Aceto-carmine 3.—Anthers at the right stage are put into a mixture of 1 part of glacial acetic acid to 9 parts of absolute alcohol, to which sufficient solution of ferric hydrate in 45 per cent. acetic has been added to color the liquid brown (the amount

varies with different objects). After some days or weeks the anthers are teased out in ordinary aceto-carmine, avoiding the use of steel instruments.

The chromosomes are usually most accurately counted in the metaphase of the second division, in dicotyledons. When the preparation is a day or two old, the cytoplasm has swollen; and a slight tap on the thin coverglass above any particular cell will usually free the cytoplasm from the cell wall, and another tap flatten it out with its contained chromosomes.

Satisfactory results have been obtained by these methods during the past year with *Datura, Canna, Antirrhinum, Linaria, Brassica, Dahlia, Secale, Asparagus, Matthiola, Phaseolus, Stizolobium, Tradescantia, Hemerocallis, Iris, Gladiolus, Zea* and *Portulaca.* The methods failed with *Œnothera* and *Rhododendron.*

The second of the above methods will probably be of the widest applicability. The preparations will keep for a week or more, if an excess of stain and of iron are avoided. The method is quicker for counting chromosomes than staining sections with iron hæmatoxylin, and in favorable cases the results may be more certain. Thus in good preparations of *Datura* over a thousand pollen-mother cells are scattered singly on one slide, many of them showing the metaphase of the second division, and some having both plates in one plane, with the chromosomes well spaced and stained a deep bluish red, while the cytoplasm is unstained. It takes certainly a modicum of patience to acquire skill with this, as with most microscopical methods.

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